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Chronic treatment with juçara (*Euterpe edulis*) fruit pulp produces antihypertensive effect and improve on baroreflex sensitivity in Spontaneous Hypertensive Rats (SHR)

Tratamento crônico com polpa do fruto de juçara (*Euterpe edulis*) produz efeito anti-hipertensivo e melhora a sensibilidade do barorreflexo em ratos espontaneamente hipertensos (SHR)

El tratamiento crónico con extracto de fruta de juçara (*Euterpe edulis*) produce un efecto antihipertensivo y mejora la sensibilidad barorrefleja en ratas hipertensas espontâneas (SHR)

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Abstract

Background: Juçara açaí is a Brazilian berry with high anthocyanidin content and high antioxidant properties. A correlation between oxidative stress, hypertension and baroreflex impairment is known, however, no previous studies investigate the effect of juçara fruit pulp on hypertension. Aim: The aim of this study was to investigate antihypertensive effect of juçara (*Euterpe edulis*) fruit pulp in SHR animals, its antioxidant capacity and influence on baroreflex sensitivity. Methods: Chemical profile was obtained by ESI(-)-FT-ICR-MS. For biological analysis, male SHR were

separated into groups: control (S), which received water; captopril (SC), which received captopril (30 mg/kg), and juçara (SJ), which received juçara pulp (4.4mL/kg). Treatments were daily performed by gavage and remained for 8 weeks. Baroreflex evaluation was made by *in bolus* injection of phenylephrine and sodium nitroprusside, after the analysis the heart was frozen (-80ºC) for biomolecular evaluations (AOPP, TBARS, SOD and catalase activity and protein expression). Results: The treatments promoted decrease in arterial pressure (S: 163.3±2.2; SC: 154.0±0.6; SJ: 149.0±3.8 mmHg) and improved the baroreflex sensitivity after nitroprusside activation. In addition, it promoted a decrease in AOPP and an increase in the activity of antioxidant enzymes. However, no changes were observed in protein expression. Conclusion: It was shown that juçara can promote cardiovascular benefits and its consume should be stimulated for prevention of hypertension.
Keywords: Anthocyanidins; Antihypertensive; Antioxidant; Atlantic açaí; Blood pressure control; Reflex pressure control.

Resumo

Introdução: O açaí juçara é uma fruta brasileira com alta concentração de antocianidinas e excelente propriedade antioxidante. A correlação entre estresse oxidativo, hipertensão e prejuízo no barorreflexo é conhecida, porém, estudos prévios investigando o efeito da polpa de juçara na hipertensão são escassos. Objetivo: O objetivo do presente estudo é investigar o efeito anti-hipertensivo da polpa do fruto de juçara (*Euterpe edulis*) em animais SHR, sua capacidade antioxidante e a influência do tratamento na sensibilidade do barorreflexo. Metodologia: O perfil químico foi obtido por ESI(-)-FT-ICR-MS. Para as análises biológicas, os ratos SHR machos foram separados em grupos: controle (S) que recebeu água; captopril (SC) que recebeu captopril (30 mg/kg) e o grupo juçara (SJ) que recebeu a polpa de juçara (4,4 mL/kg). Todos os tratamentos foram realizados diariamente por via oral (gavagem) e mantidos por 8 semanas. A avaliação do barorreflexo foi feita pela injeção *in bolus* de fenilefrina e nitroprussiato de sódio, e após as análises hemodinâmicas, os corações foram coletados e congelados (-80ºC) para as análises biomoleculares (AOPP, TBARS, atividade da SOD e catalase e expressão proteica). Resultados: Os tratamentos promoveram redução na pressão arterial (S: 163,3±2,2; SC: 154,0±0,6; SJ: 149,0±3,8 mmHg) e melhoraram a sensibilidade do barorreflexo após a ativação com o nitroprussiato de sódio. Adicionalmente, foi observado redução nos níveis de AOPP e aumento da atividade das enzimas antioxidantes. Entretanto, não foram observadas mudanças na expressão proteica. Conclusão: Nossos dados demonstram que a juçara pode promover benefícios cardiovasculares e o seu consumo deve ser estimulado para a prevenção da hipertensão arterial.
Palavras-chave: Antocianidinas; Anti-hipertensivo; Antioxidante; Açaí da Mata atlântica; Controle da pressão arterial; Controle reflexo da pressão.

Resumen

Introducción: Juçara açaí es una fruta brasileña con alta concentración de antocianidinas y excelentes propiedades antioxidantes. La correlación entre el estrés oxidativo, la hipertensión y el daño barorreflejo es conocido, sin embargo, los estudios previos que investigan el efecto de la pulpa de juçara en la hipertensión son escasos. Objetivo: El objetivo del presente estudio es investigar el efecto antihipertensivo de la pulpa del fruto de juçara (Euterpe edulis) en animales SHR, su capacidad antioxidante y la influencia del tratamiento en la sensibilidad barorrefleja. Metodología: El perfil químico se obtuvo por ESI(-)-FT-ICR-MS. Para los análisis biológicos, las ratas macho SHR se separaron en grupos: control (S) que recibió agua; captopril (SC) que recibió captopril (30 mg/kg) y el grupo juçara (SJ) que recibió pulpa de juçara (4,4 mL/kg). Todos los tratamientos se realizaron diariamente por vía oral (gavage) y se mantuvieron durante 8 semanas. La evaluación del barorreflejo se realizó mediante inyección en bolo de fenilefrina y nitroprusiato de sodio, y después del análisis hemodinámico, los corazones se recolectaron y congelaron (-80ºC) para análisis biomolecular (AOPP, TBARS, SOD y actividad de catalasa y expresión de proteínas). Resultados: Los tratamientos promovieron una reducción de la presión arterial (S: 163,3±2,2; SC: 154,0±0,6; SJ: 149,0±3,8 mmHg) y mejoraron la sensibilidad barorrefleja después de la activación con nitroprusiato de sodio. Además, se observó una reducción en los niveles de AOPP y un aumento en la actividad de las enzimas antioxidantes. Sin embargo, no se observaron cambios en la expresión de proteínas. Conclusión: Nuestros datos demuestran que la juçara puede promover beneficios cardiovasculares y se debe fomentar su consumo para prevenir la hipertensión arterial.
Palabras clave: Antocianidinas; Antihipertensivo; Antioxidante; Açaí de la Mata Atlántica; Control de la presión arterial; Control de la presión refleja.

1. Introduction

Fruits are considered a rich source of bioactive compounds, with a high number of polyphenols and other compounds that confer them a great antioxidant capacity (Batista et al. 2016; dos Reis et al. 2018; Barroso et al. 2019). Among the fruits with such potential, açai berries are one of the main sources of bioactive compounds with biological activity (Da Costa et al. 2017; Barroso et al. 2019).

Brazil presents a high amount of species of açai berries, which includes the specie *Euterpe edulis Martius* (*E. edulis*). *E. edulis* is a palm that produces small dark purple fruits, with a high amount of anthocyanidins (Inácio et al. 2013; Schulz et al. 2015, 2016). It is popular known as juçara palm, juçara or Atlantic forest açai (Schulz et al. 2016). Since the sixties, juçara is the most extracted palm from Atlantic forest, due to the exploration of palm heart. Because of it, the specie has been included in the Brazilian list of endangered species (Schulz et al. 2016). Thus, the exploration of fruits is an important option to preserve the palm.

Polyphenols, which includes anthocyanidins, promote beneficial effects on cardiovascular system and could also decrease the risk of developing cardiovascular diseases, CVD (Cassidy et al. 2016; Cassidy 2018). CVD are the leading cause of death in the modern world and include hypertension (Benjamin et al. 2018). However, even with traditional treatment for hypertension being available, the disease is continuously growing around the world (WHO 2013; WHO - World Health Organization 2013).

Hypertension involves the dysregulation of humoral and neural control of cardiovascular system (Oparil et al. 2018), being the increase of Reactive Oxygen Species (ROS) and the impairment of baroreflex sensibility important to the dysregulation of such a control, respectively.

The high amount of ROS produced in vessels leads to the increase in peripheral vascular resistance, thus contributing to increased arterial pressure (Rodrigo et al. 2011; Virdis et al. 2011). Such effect could be specially attributed to the decrease in nitric oxide (NO) bioavailability, which is an important vasodilator agent (Rodrigo et al. 2011). The high amount of ROS, specially superoxide anions, may lead to a decrease in the bioavailability of NO by the generation of peroxynitrite. That decrease in NO bioavailability has a central role in endothelial dysfunction and the development of hypertension (Panza et al. 1993; Oparil et al. 2018). Usually, ROS are scavenged by antioxidant system, specially by superoxide dismutase (SOD) and catalase (Cat) enzymes (do Vale & Tirapelli 2019).

Hypertension treatment is based on the regular use of medicines and changes in the lifestyle (Whelton et al. 2017). Also, such a change in lifestyle that includes physical activity, decrease of salt intake and addition of bioactive compounds in diet are highly encouraged (Cassidy et al. 2016; Whelton et al. 2017). Food can act as adjuvant, leading to a synergistic effect if combined with traditional treatments, thus promoting better results for patients (Cassidy et al. 2016; Yuan et al. 2016). Previous studies have shown the antihypertensive effect of isolated flavonoids and that flavonoid-enriched food present positive results, which are associated with their vasodilator/antioxidant capacity and a multi target action (Grassi et al. 2013).

Based on the above, the hypothesis of the present study is that chronic treatment with juçara fruit extract promotes a decrease in blood pressure and improves baroreflex sensitivity in spontaneously hypertensive animals (SHR) by decreasing oxidative stress, thus suggesting a new and potential use of this endangered species. Thus, the aim of this study was to determine the antihypertensive effect of chronic treatment with juçara fruit extract and its impact on baroreflex and oxidative stress in SHR animals.

2. Methodology

2.1 Extract preparation

Juçara fruits were collected in Santa Teresa – ES at the Biological reserve Santa Lucia and were assigned by INCAPER (Capixaba Institute for Research, Technical Assistance and Rural Extension). Fruits were cleaned and frozen at -20 ºC prior to their use. The plant material was identified by Ms Solange Zanotti Schneider and a voucher specimen was deposited at the herbarium of Universidade Vila Velha (number 2396). A manual extraction process was performed, where the fruits were washed and maintained in water for 30 minutes (40º C). After that, 250mL of water was used to perform the extraction. The fruit extract was freeze dried and keep at -80ºC until use (Fadden et al. 2008).

2.2 Chemical characterization by negative ion-mode electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry ESI(-)-FT-ICR-MS

A sample of juçara extract (1mg) was analyzed by ESI ESI(−)-FT-ICR-MS. The sample was mixed with 1 μL of NH_4OH and injected in the FT-ICR MS spectrometer (model 9.4 T Solarix, Bruker Daltonics, Bremen, Germany). The spectra were obtained in negative mode from 200-2000 m/z. The analysis was performed at 2.0 bar of pressure for nebulizer gas, capillary voltage of 2.5 kV and temperature of 280ºC. The scans were accumulated at each 0.001s and a total of 100 scans were obtained. Mass spectra were acquired and processed using Compass Data Analysis software (Bruker Daltonics, Bremen, Germany) and molecular formulae were obtained using ChemSpider database software (www.chemspider.com).

2.3 Animals

Male Spontaneously Hypertensive Rats (SHR) of eight weeks of age and weight between 200-250g were used. They were provided by Laboratório de Acompanhamento Experimental do Complexo Biopráticas – UVV. The animals were kept in collective insulators (Rack IVC Individually Ventilated Caging Alesco©) with controlled humidity, temperature and dark-light cycle. The access to food and water was *ad libitum* (Standard feed Probiotério, Moinho Primor, S.A.). All the procedures were performed in agreement with National Institute of Health (Garber et al. 2010) and were approved by ethics committee for animal use of University Vila Velha (CEUA-UVV. #447/2017).

2.4 Experimental protocol

The animals were separated in three groups (n=6 each). Control group (S), received purified water daily, by gavage; Captopril group (SC) received captopril as a standard antihypertensive drug at a dose of 30mg/kg (daily, by gavage) (Quilley et al. 1987); Juçara group (SJ) received the extract of juçara fruit at a dose of 4.4 mL/kg. The daily dose of juçara was calculated based on a human consume of 150mL of juçara açai per day (Reagan-Shaw et al. 2008).

The animals were weighted at the beginning (IBW) and at the end (FBW) of treatment period and the weight gain was determined by the ratio between them (IBW/FBW). All the treatments were performed for eight weeks (Da Costa et al. 2017).

2.5 Hemodynamic measurement and baroreflex evaluation

After the treatment period, the animals were intraperitoneally anesthetized with a mixture of ketamine and xylazine (100/10 mg/kg; Dopalen®, Ceva, Paulínia – SP; Sedomin®, Konig, Mairinque – SP). Under deep anesthesia, the animals had the femoral arteria and vein cannulated (Andrade et al. 2008). At least 24 hours after the procedure, the mean arterial pressure (MAP) and heart rate (HR) were evaluated in the animals as described previously (Brasil et al. 2014). After a 30-min of stabilization period, the baroreflex sensitivity was evaluated (Ai et al. 2010). In resume, MAP and HR were controlled at a beat level as a response of the application of randomized doses of phenylephrine (25-100 μg/kg) or sodium nitroprusside (45-180 μg/kg). The regression coefficient (slope of curves) expressed in beat per minute ($bpm.mmHg^{-1}$) was used as baroreflex sensitivity index (BRS) (El-Mas et al. 2001).

2.6 Tissue collection and organ hypertrophy evaluation

After hemodynamic evaluations, the animals were euthanized and the heart, liver and kidney were excised, cleaned, dried and weighted. The organ weight was normalized by tibial length (g/cm) and used as parameter of organ hypertrophy. Additionally, the heart was frozen (-80ºC) for later biochemical analysis.

2.7 Protein quantification

The protein content was determined by the Bradford method (Bradford 1976). Briefly, the heart homogenate (10µL) was mixed with Bradford reagent (190µL) and, after 5-min of stabilization, the absorbance was measured at 595 nm (Filter Max F5 Multi-Mode Microplate Readers, Molecular Devices, California, EUA).

2.8 Determination of oxidation products

The protein and lipid oxidation were determined by AOPP and TBARS methods.

For AOPP evaluation, the method of Witko-Sarsat et al. (Witko-Sarsat et al. 1996) was used. The heart homogenate was mixed with potassium iodide (KI, 1.16M) and acetic acid. After continuous agitation, the absorbance was measured at 340 nm (Filter Max F5 Multi-Mode Microplate Readers, Molecular Devices, California, EUA). The result was determined using a standard curve with chloramine T (100-1000 µM) and was expressed as µM de chloramine T/mg de protein.

To determine TBARS, the cardiac homogenate (200µL) was mixed with thiobarbituric acid (200µL). After heating (100ºC) for two hours and a complete cooling, the absorbance was measured at 532 nm (Filter Max F5 Multi-Mode Microplate Readers). A standard curve with malondialdehyde was obtained (MDA; 0.5-25 µM) and the results were expressed as µM of MDA/mg de protein.

2.9 Antioxidant enzymes activity

The antioxidant enzymes superoxide dismutase (SOD) and catalase (Cat) were determined as follows:

For SOD evaluation, the method proposed by Of et al., (1972) was used. The reaction was performed by the addition of 1 mL of carbonate buffer (0.2 M, pH 10.2), 0.8 mL of KCl (0.015M), 0.1 mL of sample homogenate and water up to 3mL. The reaction was initiated by addition of 0.2mL of epinephrine (0.025 M). The absorbance was recorded at 480nm for 1 minute. The results are expressed as USOD/mg protein, and 1 USOD was consider the amount of enzyme sufficient to decrease in 50% the epinephrine autoxidation.

Catalase activity was determined as described by Aebi (Aebi 1984) with minor modifications. The sample homogenate (60µL) was mixed with phosphate buffer (3mL; 0.050 M, pH 7.4) and 40µL of H_2O_2 (0.066 M). The variations in sample absorbance was monitored at 240nm for 1 minute. The results were expressed as peroxide extinction coefficient (ΔE.min/mg protein).

2.10 Western blot

The heart tissue was homogenized in lysis buffer (100 mmol/L NaCl, 50 mmol/L Tris, 5 mmol/L EDTA 2Na, 50 mmol/L $Na_4P_2O_7.10H_2O$, 1 mmol/L $MgCl_2$, 0.3% Triton x-100 and 0.5% of sodium deoxycholate; pH = 8) with protease inhibitor (Sigma fast, Sigma, EUA). An amount of 50mg of protein was mixed with Laemmle buffer (5× 2M Tris, pH = 6.8, 20% glycerol, 30% SDS, 25% 2-mercaptoethanol and 0.1% Bromophenol Blue) followed by separation in acrylamide gel (SDS-Page). The protein was transferred to a PVDF membrane (Polyvinylidene difluoride) and blocked (Tris 20 mM, NaCl 150 mM, Tween 0.05%, non-fat milk 4%, pH 7.6). The following primary antibodies were used: anti-SOD1 (1:1000 anti mouse, Santa Cruz Biotechnology®, Texas – USA), anti-catalase (1:1000 anti-mouse, Santa Cruz Biotechnology®, Texas – USA) or anti-GAPDH (1:1000, anti-mouse, Santa Cruz Biotechnology®, Texas – USA). After incubation, the membranes were washed and incubated with the secondary antibody conjugated with alkaline phosphatase (1:5000, anti-mouse, Sigma Aldrich®, Missouri – USA). The immunoreactive bands were shown by the reaction with NBT/BCIP system (Invitrogen, USA). The band intensity was determined using the software Image J® (National Institutes of Health, Bethesda, MD, EUA) and expressed in arbitrary units. GAPDH was used as normalized protein.

2.11 Statistical analysis

The normality of data was determined by Kolmogorov-Smirnov normality test. The difference among the means was determined using one way-analysis of variance (ANOVA) followed by Tukey post hoc test. The significance was accepted when $p<0.05$. Data were represented as mean ± standard error of mean (SEM).

3. Results

3.1 Chemical evaluation

ESI(–)FT-ICR mass spectra were obtained from juçara fruit extract of *E. edulis*. The high resolution and accuracy of FT-ICR MS allowed the structural identification of 12 compounds, where their molecular formulae, *m/z* values, double bound equivalents (DBEs), and mass errors (ppm) are shown in Table 1.

Table 1. ESI(-)-FIT-ICR-MS results for juçara extract.

Compound	m/z (exp.)	Molecular Formula $[M–H]^-$	DBE	Error (ppm)	Phytochemical group	Putative Identification
1	191.0563	$C_7H_{11}O_6$	2	-1.00	cyclitol	Quinic acid
2	193.03548	$C_6H_9O_7$	2	-0.56	carbohydrate	Galacturonic acid
3	215.03304	$C_6H_{12}ClO_6$	-	-1.15	carbohydrate	Glucose
4	255.23332	$C_{16}H_{31}O_2$	1	-1.42	fatty acids	Palmitic Acid
5	283.26469	$C_{18}H_{35}O_2$	1	-1.55	fatty acids	Stearic acid
6	315.07268	$C_{13}H_{15}O_9$	6	-1.66	Phenol acid	Gentisoylglucoside
7	341.1093	$C_{12}H_{21}O_{11}$	2	-1.07	carbohydrate	Sacarose
8	353.10951	$C_{13}H_{21}O_{11}$	3	-1.63	carbohydrate	Methyl 6-deoxy-3-O-β-D-galactopyranuronosyl-α-L-mannopyranoside
9	359.09909	$C_{15}H_{19}O_{10}$	6	-2.00	phenol	1-O-(4-Hydroxy-3,5-dimethoxybenzoyl)-β-D-glucopyranose
10	479.12087	$C_{22}H_{23}O_{12}$	11	-2.86	anthocyanin	Petunidin-3-O-β-glucopyranoside
11	533.17308	$C_{19}H_{33}O_{17}$	3	-1.41	carbohydrate	D-glycero-D-manno-Heptopyranosyl-(1->6)-D-glucopyranosyl-(1->2)-D-glucopyranose
12	603.17984	$C_{22}H_{35}O_{19}$	5	-3.38	carbohydrate	α-D-GlcpA4Me-(1->2)-β-D-Xylp-(1->4)-β-D-Xylp-(1->4)-β-D-Xylp

Source: Authors.

Carbohydrate (mono, di, tri, tetra and oligosaccharides) was the main phytochemical group identified, which is expected considering the definition of fruit pulp as 'the unfermented, not concentrated, not diluted, pulpy fruit product obtained by means of appropriate technological process, having a minimum of total solids obtained from the edible part of fruit' (Brasil 2000).

The other identified phytochemical groups were cyclitol (compound 1, $[C_7H_{11}O_6]^-$ ion, of *m/z* 191, quinic acid), fatty acids (compounds 4 and 5, $[C_{16}H_{31}O_1]^-$ and $[C_{18}H_{35}O_2]^-$ ions, m/z 255 and 283, palmitic and stearic acids), phenol and phenolic acids (compound 6, $[C13H15O9]^-$, *m/z* 315, gentisoylglucoside, compound 9, $[C15H19O10]^-$, *m/z* 359, 1-O-(4-Hydroxy-3,5-

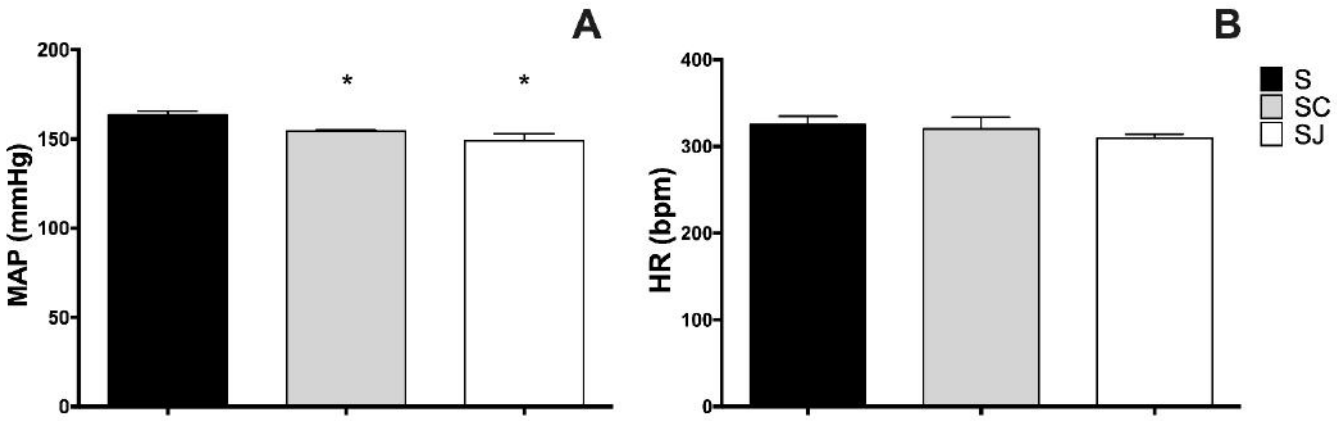
dimethoxybenzoyl)-β-D-glucopyranose) and anthocyanin (compound 10, $[C_{18}H_{35}O_2]^-$ ions, m/z 479, Petunidin-3-O-β-glucopyranoside).

Compound 2, $[C6H9O7]^-$ ion, of *m/z* 193, was identified as galacturonic acid, a monosaccharide. The glucose (monosaccharide) molecule was identified as chlorine adducts, producing the compound 3, $[C_6H_{12}O_6 + Cl]^-$ ion. Compounds 7 and 8, $[C12H21O11]^-$ and $[C13H21O11]^-$ ions, of *m/z* 341 and m/z 353, were identified as disaccharides sucrose and methyl 6-deoxy-3-O-*β*-d-galactopyranuronosyl-*α*-l-mannopyranoside. Compounds 11 and 12, $[C19H33O17]^-$ and $[C22H35O19]^-$ ions, *m/z* 533 and 603, respectively, were identified as tri and tetrasaccharide D-glycero-D-manno-Heptopyranosyl-(1->6)-D-glucopyranosyl-(1->2)-D-glucopyranose and α-D-GlcpA4Me-(1->2)-β-D-Xylp-(1->4)-β-D-Xylp-(1->4)-β-D-Xylp, respective.

3.2 Hemodynamic measurement and baroreflex evaluation

The chronic treatment with juçara extract shows an antihypertensive effect as demonstrated by the decrease of mean arterial pressure (Figure 1. Panel A. S: 163.3±2.2; SC: 154±0.6; SJ: 149.1±3.8 mmHg) without changing heart rate (Figure 1. Panel B. S: 325.6±9.8; SC: 320.2±13.9; SJ: 309.3±4.6 bpm). Such result was like those obtained with captopril, the standard drug used to treat hypertension.

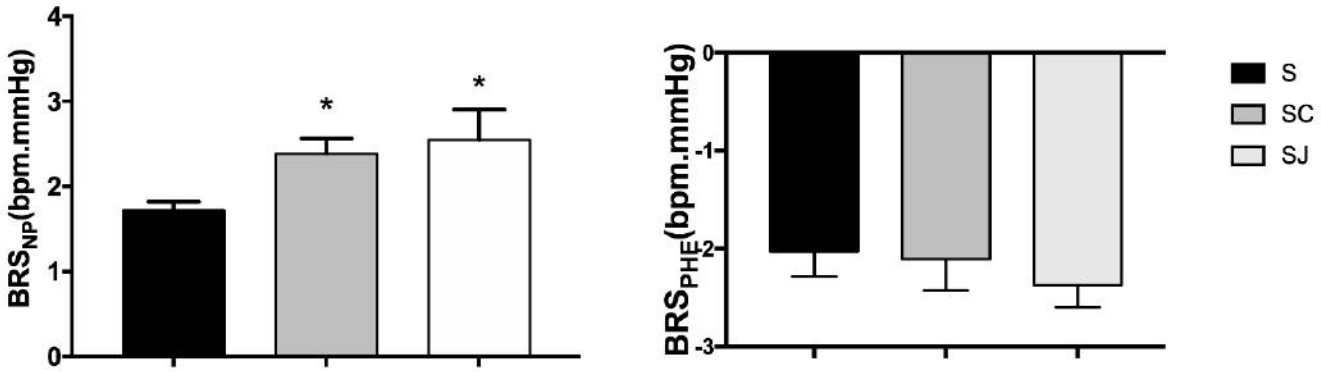
Figure 1: Results of evaluation of mean arterial pressure (MAP) and heart rate (HR) after chronic treatment with juçara fruit extract in SHR animals. **Panel A.** Evaluation of mean arterial pressure. **Panel B:** Values of heart rate in different treatment groups. S: SHR control animals; SC: SHR animals treated with captopril (30mg/kg/day); SJ: SHR animals treated with juçara fruit extract (4.4mL/kg/day). Data are present as mean ± standard error of mean (S.E.M.). Results were evaluated by ANOVA followed by Tukey's post hoc test. *p<0.05 compared to S.



Source: Authors.

The antihypertensive activity was accompanied by an improvement of baroreflex sensitivity, when using sodium nitroprusside (Figure 2. Panel A. BRS_{NP} S: 1.718±0.100; SC: 2.385±0.178; SJ: 2.542±0.363 bpm/mmHg) but no changes were observed when phenylephrine was used (Figure 2. Panel B. BRS_{PE} S: -2.028±0.256; SC: -2.105±0.322; SJ: -2.370±0.228 bpm/mmHg), and similar results were obtained using captopril.

Figure 2. Results of baroreflex sensitivity after activation with sodium nitroprusside (Panel A) or phenylephrine (Panel B) in different groups of treatment. Juçara fruit extract promotes improvement of baroreflex sensitivity when activated by sodium nitroprusside (Panel A), however, no changes were observed after phenylephrine. Data are present as mean ± standard error of mean (S.E.M.). Results were evaluated by ANOVA followed by Tukey's post hoc test. *p<0.05 or compared to S.

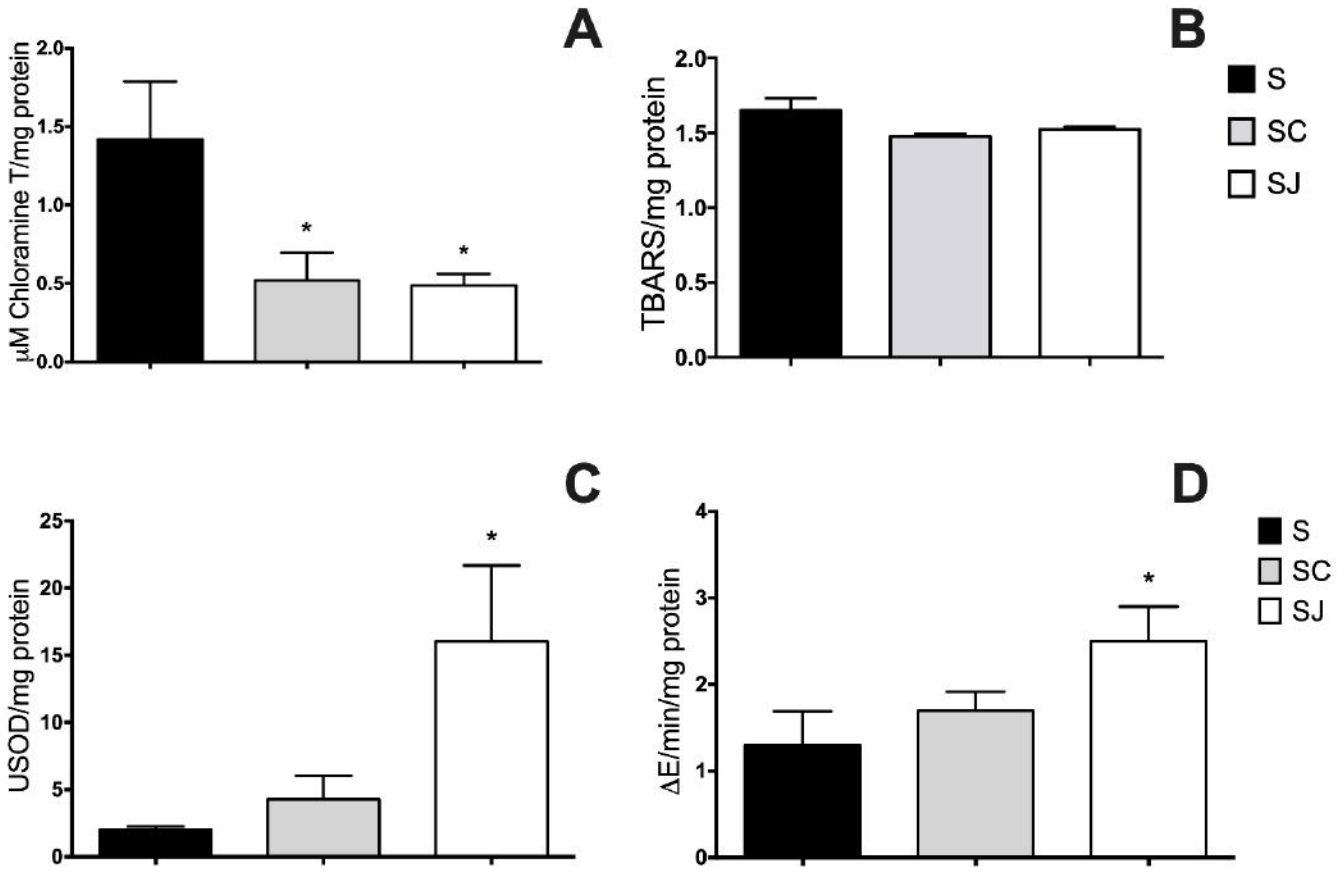


Source: Authors.

3.3 Oxidative stress

The evaluation of oxidative stress in cardiac tissue has been performed. The products of protein oxidation decreased in the group that received juçara (Figure 3, Panel B; S: 1.388±0.289; SC: 0.519±0.137; SJ: 0.487±0.075 μM chloramine T/mg of protein) which indicates a decrease in oxidative stress. However, no difference was observed in lipid peroxidation (Figure 3, Panel B; S: 1.683±0.076; SC: 1.476±0.014; SJ: 1.588±0.033 MDA/mg of protein). Antioxidant activity of juçara treatment was followed by an increase in SOD (Figure 3, Panel S: 2.006±0.258; SC: 4.275±1.764 SJ: 16.02±5.655 USOD/mg of protein) and Catalase (Figure 3, Panel D; S: 1.3±0.39 SC: 1.7±0.22 SJ: 2.5±0.4 DE.min.mg of protein) enzyme activity.

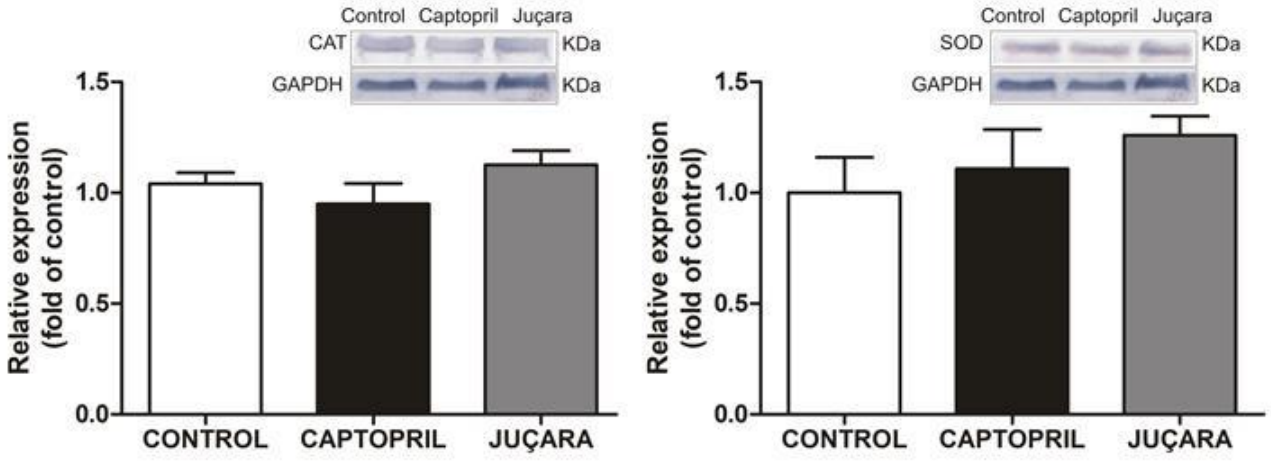
Figure 3. Quantitative determination of products of protein oxidation (AOPP) and thiobarbituric reactive substances (TBARS) and activity of antioxidant enzymes (SOD and Catalase). Chronic treatment with juçara extract promotes decrease in AOPP species (Panel A) and no changes in TBARS products are observed (Panel B). Also, there was an increase in SOD and Catalase activity (Panel C-D). Data are present as mean ± standard error of mean (S.E.M.). Results were evaluated by ANOVA followed by Tukey's post hoc test. *p<0.05 compared to S;



Source: Authors.

To determine if the observed increase in enzyme activity was promoted by an improvement in SOD and Catalase protein expression, the western blot quantification has been performed. As can be seen in Figure 4, no changes were observed in protein expression of both SOD (S: 1.00±0.16; SC: 1.11±0.18; SJ: 1.26±0.09 relative expression) and Catalase (S: 1.04±0.05; SC: 0.95±0.09; SJ: 1.13 ± 0.06 relative expression) enzymes.

Figure 4: Antioxidant enzymes expression by western blot. Treatment with juçara extract did not improve protein expression of both SOD and Catalase enzymes. Data are present as mean ± standard error of mean (S.E.M.). Results were evaluated by ANOVA followed by Tukey's post hoc test. *p<0.05 compared to S; #p<0.05 compared to SC group.



Source: Authors.

3.4 Weight measurements

Table 2 show the results for weight measurements. The treatment with juçara was able to promote a decrease in cardiac hypertrophy, similarly to captopril. However, no changes in body weight gain and kidney and liver hypertrophy was observed among the groups.

Table 2. Results of weight measurements for SHR animals treated with juçara fruit or captopril.

Results	Groups		
	S	SC	SJ
Body weight gain (IW/FW)	1.424±0.016	1.391±0.002	1.383±0.011
Liver weight/tibial length (g/cm)	3.093±0.224	3.358±0.176	3.050±0.110
Kidney weight/tibial length (g/cm)	0.3244±0.0124	0.3298±0.0123	0.3110±0.0129
Heart weight/tibial length (g/cm)	0.3008±0.0132	0.2673±0.008*	0.2774±0.006*

Data are present as mean±standard error of mean (SEM). The results were evaluated by ANOVA followed by Tukey's post hoc test. *p<0.05 compared to S group; Source: Authors.

4. Discussion

The main result of the present study was that juçara fruit extract promotes antihypertensive effects associated with the improvement in baroreflex sensitivity for spontaneously hypertensive rats (SHR). The result can be attributed, at least in part, to antioxidant properties of the fruit extract.

In the present study, it was shown that the intake of juçara fruit extract promotes decrease in blood pressure in SHR animals similar to Captopril treated group (SC), a well-known antihypertensive agent with a better therapeutic result (Whelton et al. 2017).

Such an antihypertensive effect can be attributed to the rich chemical composition of the fruit, with a large number of polyphenols, flavonoids and anthocyanins (Schulz et al. 2016; Barroso et al. 2019). Polyphenols are related with several positive effects, including decrease in oxidative stress (Borges et al. 2011; Bicudo et al. 2014; Schulz et al. 2015).

Juçara fruit is rich in anthocyanins (Borges et al. 2011; Vieira et al. 2013) and, in the current study, petunidin-3-O-β-

glucopyranoside was found, in accordance with a previous report (Brito et al. 2007), and was related with enhanced antioxidant activity (Kähkönen & Heinonen 2003). It is well known that the intake of natural products rich in polyphenols could be an alternative to treat and prevent hypertension (Hügel et al. 2016), specially by its capacity to defense the body against ROS, which occurs specially through its reaction with free radicals promoting the *scavenger* of species like oxygen peroxide. Also, polyphenols can improve the endogen antioxidant systems (Cardoso et al. 2018).

The antihypertensive effect by plants of *Euterpe* genus have been previously reported in SHR animals (Cordeiro et al. 2015) and in a renovascular model of hypertension (Da Costa et al., 2012). However, these studies have investigated the specie *E. Oleracea Mart*., popularly known as Amazonia açaí (Heinrich et al. 2011; Yamaguchi et al. 2015). Gale et al. (2014) evaluated the impact of Amazonia açaí consume on the health of individuals and observed an acute decrease in systolic blood pressure (SBP) six hours after juice intake. On the other hand, Udaniet al. (2011) evaluating the consume of 100 mg of Amazonia açaí/day for four weeks, did not observed such a decrease of blood pressure. Nevertheless, it is important to highlight that no study evaluating the consume of juçara fruit extract in a model of essential hypertension, in animals or humans, were found. So, the present study was the first to show the antihypertensive effect of *E. edulis* in SHR animals.

Baroreflex is responsible for the control of blood pressure at a heartbeat level and the decrease in its sensitivity is remarkable in hypertension (Head, 1995; Meyrelles et al., 1998; Vasquez et al., 2012, Whelton et al, 2018) and is related to sudden death (Tsai et al. 2019) and others CVD (Grans et al. 2014). In our study, the decrease in MAP was associated with an improvement in BRS in animals of SJ group, similar to captopril, which is recognized as an enhancer of baroreflex sensitivity in SHR (Cheng et al. 1989). Thus, our results show that treatment with juçara can lead to improved baroreflex gain, indicating that the extract can promote a good blood pressure control.

The essential hypertension is considered a multifactorial disease with an important participation of ROS production on its development (Rodrigo et al. 2011; Guzik & Touyz 2017). The increase in ROS leads to depletion in endothelial nitric oxide (NO), which is an important vasodilator agent responsible for the control of peripheric arterial resistance (Bernardes et al., 2014; Cunha et al., 2005; Judkins et al., 2010; Tain & Huang, 2014; Vignon-Zellweger et al., 2014).

Our results show an indirect decrease in oxidative stress, through a reduction of AOPP, and an increase in the activity of antioxidant enzymes. Our results corroborate those found by Freitas et al., (2017) that showed an increase in SOD and Catalase activity in *wistar* rats that intake cafeteria diet and received *E. edulis* for 20 days. However, de Castro et al., (2014) evaluating the impact of *E. edulis* and physical activity on ApoE deficient mice, observed that the supplementation with juçara did not show improvements in the antioxidant enzymes activity. The discrepant results observed in the two studies can be attributed to different protocols and doses employed

In the present study, we observed a decrease in oxidative stress products, with an increase in SOD and Catalase activity, that was not accompanied by an increase in protein expression, as revealed by western blot. Polyphenols can act directly with free radicals present in tissue, or even by changes in molecular mechanisms such as activation of erythroid transcription factor 2 (Nrf-2), which is an important pathway of transcription of antioxidant enzymes genes (Tresserra-Rimbau et al. 2018).

Additionally, chronic treatment with juçara promotes a decrease in cardiac hypertrophy similar to captopril, the standard drug. Cardiac hypertrophy is a pathophysiological condition that is related with the development of comorbidities like heart failure (Grassi et al. 2006; Tsai et al. 2019). Hypertension may also contribute to cardiac hypertrophy development (Mancia et al. 1982; Whelton et al. 2018), so the decrease in blood pressure promoted by juçara may be the explanation, at least in part, for the amelioration of that parameter in the investigated animals. Strengthening the importance of the relationship between increased MAP and cardiac hypertrophy, the literature demonstrates that in the SHR hypertension model, hypertension precedes the onset of cardiac hypertrophy (Li et al. 2019). Additionally, high blood pressure is also related to cardiac hypertrophy because it increases the production of ROS, which together can lead to heart failure (Rababa'h et al. 2018). According to Mengal et al.,

(2016), the administration of antioxidants in renovascular hypertensive animals reduces the oxidative stress, restores the baroreflex sensitivity, and decrease the MAP. In this way, the use of juçara extract could be a beneficial product to prevent and / or reverse these changes and contribute to preventing the heart failure associated with hypertension.

Additionally, the investigated animals did not present weight loss during the treatment, which indicates safety in the use of juçara (Mariz et al. 2012). A previous study performed by Felzenszwalb et al., (2013) showed that juçara pulp extract is safe up to a daily intake of 22.5 mg/kg. In humans, the dose of 300mL of juçara açaí did not promote negative changes in peripheral blood cells. Additionally, no changes were observed in weight after treatment.

In summary, chronic treatment with juçara fruit extract promotes decrease in mean arterial pressure with improvement of baroreflex sensitivity and a decrease in heart hypertrophy in SHR animals. Additionally, it was observed a reduction in AOPP values associated with an increase in SOD and catalase activity. No changes were observed in antioxidant enzymes expression by western blotting.

5. Conclusion

It is possible to conclude that juçara promotes an antihypertensive effect through a decrease in reactive oxygen species. This is the first study to demonstrate this for juçara specie in hypertension model that resembles essential hypertension in humans (SHR), which indicates that the consume of that pulp promotes health benefits and could be used by patients as a nutraceutical. Such important results may contribute to a new use of juçara and thus contribute to the preservation of an endangered species

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